# CONDUCTIVE TISSUE IN *CERATOPHYLLUM DEMERSUM* (CERATOPHYLLACEAE)

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ABSTRACT

Using scanning electron microscopy (SEM) and light microscopy, we attempted to establish if xylem and phloem occur in *Ceratophyllum* stems; roots are lacking in the genus. In larger stems, a central core of elongate cells contains scattered sieve tube elements with simple sieve plates; the sieve tubes are associated with companion cells. Sieve tubes are not organized into phloem strands. The central core of main and branch stems contains elongate cells, some of which lack starch and tannins. These cells lack any evidence of the annular or helical thickenings of secondary wall material characteristic of primary xylem in other vascular plants, and therefore xylem cannot be claimed for *Ceratophyllum*. Absence of xylem in *Ceratophyllum* is interpreted as secondary, a loss related to adaptation to the submersed habit.

RESUMEN

Empleando la microscopía electrónica de barrido y la microscopía óptica, hemos intentado establecer si existe xilema y floema en los tallos de *Ceratophyllum*; no existen raíces en el género. En los tallos más grandes, un núcleo central de células alargadas contiene elementos cribosos dispersos; estos elementos cribosos tienen placas cribosas simples y están asociados con células compañeras. Los elementos cribosos no se organizan en paquetes de floema. El núcleo central de los tallos principales y laterales contiene células alargadas, y en algunas falta el almidón y los taninos. Estas células no tienen engrosamientos espiralados o anillados en la pared secundaria característicos del xilema primario en otras plantas vasculares, y no puede decirse que haya xilema en *Ceratophyllum*. La ausencia de xilema en *Ceratophyllum* se interpreta como secundaria, una pérdida relacionada con la adaptación al hábito sumergido.

## INTRODUCTION

Ceratophyllaceae have recently attracted attention because of shifts in concepts of their evolutionary relationships. Earlier phylogenetic schemes placed them nearer such families of aquatic dicotyledons as Cabombaceae (Ito 1987) or Nelumbonaceae (Thorne 1987); Les (1988) reviewed placements by various authors. Cladistic analyses based on macromorphological data have placed Ceratophyllaceae near Nymphaeaceae, in a basal position in dicotyledons (Les 1988). Cladistic analysis of *rbc*L data (Les et al. 1993; Qiu et al. 1993) confirmed this idea, and locates Ceratophyllaceae as the outgroup of all other angiosperms. Conceding that Ceratophyllaceae may

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contain both specialized and primitive features, knowledge of any feature in the family is important in view of the phyletic placement now accorded Ceratophyllaceae.

Klercker (1885) showed that *Ceratophyllum* has sieve-tube elements with associated companion cells ("cellules adjunctives"), but reported no xylem cells. Jones (1931) found sieve-tube elements and companion cells; he claimed presence of phloem parenchyma, and also believed that "some of the sieve tubes show evidence of transformation into air spaces. This degeneration is particularly evident in stems in which the aerenchyma is strongly developed." Jones further claimed that "the xylem is so degenerated that there is in reality very slight evidence of this tissue. There are two kinds of cells composing the xylem. One is larger than the other in diameter and has thicker walls. It is probably a reduced vessel. The other is of the xylem parenchyma type. There is abundant evidence that the reduced vessel-like cells are becoming parenchymatous." Jones cited presence of tannins, chloroplasts, and starch in the "reduced vessel-like" cells surrounding the central canal. In his legend for a transection of a mature stem, Jones's conclusions are somewhat ambiguous. He believed that there is "transformation of some xylem vessels into parenchyma cells," but also considers that "it is perhaps more appropriate to designate all the tissue inside the phloem as pith since the cells appear to be assuming the function of parenchyma and in a few cases, even that of chlorenchyma. Furthermore they are quite similar to parenchyma cells in appearance." Jones (1931) failed to find any evidence of lignin in cells.

The purpose of our study is to reinvestigate the nature of conductive tissue in *Ceratophyllum*, using both light microscopy and SEM. Is the phloem clearly referable to that concept, as Jones claimed? Does the central tissue of stems (either main or branch stems) deserve the designation of xylem, or is it merely pith? Is there any evidence that cells are "reduced" vessels or tracheids? Is reduction or absence of xylem related to adaptation to the aquatic habitat, as Jones (1931) claimed?

## MATERIALS AND METHODS

Our materials of *Ceratophyllum demersum* L. were collected from two sites, both in Texas, during September, 1995. The primary collection site was Aquarena Springs, the headwaters of the San Marcos River in San Marcos, Texas. In this site, where clear water of constant temperature (21° + 1°C) emerges from a limestone aquifer, *C. demersum* is common, with several individuals anchored at depths of more than 5 m. Additional collections were obtained from Toledo Bend Reservoir, Sabine County in eastern Texas.

Stems were fixed in 50% ethanol. Portions were dehydrated according

to the tertiary butyl alcohol method (Johansen 1940), embedded in paraffin, and sectioned on a rotary microtome. Some sections were stained with a safranin-fast green series corresponding to Northen's modification of Foster's tannin acid-ferric chloride method (Johansen 1940). Other sections were mounted on aluminum stubs much as paraffin sections would be affixed to glass slides; these sections were then cleansed of paraffin with xylem, sputter coated, and then observed with a Bausch and Lomb Nanolab SEM.

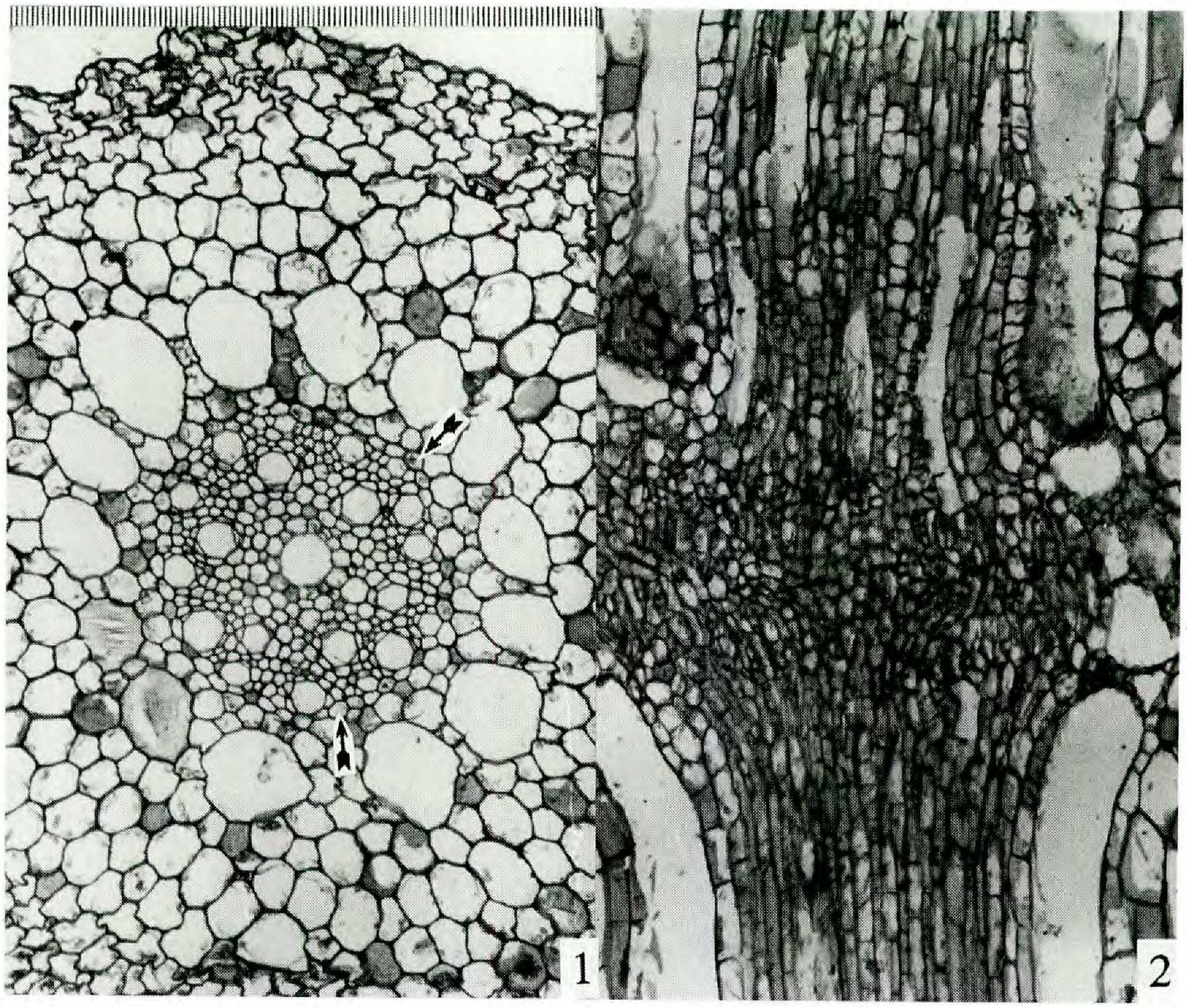
## ANATOMICAL RESULTS

Transections of the main stem (Fig. 1) have a cortical region delimited by a circle of rather large cylindrical air canals, appreciably larger than the parenchyma cells of the cortex. Internal to the cylinder of air canals is what Jones (1931) considered the vascular core. Although we did not find any histological differentiation of the cells at the periphery of the core, Jones (1931) identified an endodermis in labels of his figures. Presumably he considered the narrowest cells at the periphery of the vascular core to be endodermis (Fig. 1). Within the vascular core, there are air canals (Fig. 1, center). Longisections show that these are air canals rather than enlarged cells. One of these may be placed centrally in the core (Fig. 1). This designation is in agreement with Jones (1931), who, however, thought of these stelar air canals as modified sieve tubes: "the erstwhile sieve tubes are being modified for this purpose." In general, the sieve-tube elements are located in the outer portion of the vascular core, as one might expect in a dicotyledonous stem.

If one views a longisection of a *Ceratophyllum* stem, one sees that at nodal regions, cells of the central core are irregular in orientation, and the vertical air spaces are interrupted by a nodal plate (Fig. 2). The nodal region as seen in transection (Fig. 3) illustrates that strands of elongate cells extend from the vascular core into the branches that occur in verticillate fashion at the nodes.

Examining with SEM the cells that lead from the central core into the branches, one finds that most of these cells are elongate, or prosenchymatous, in shape (Fig. 4). Many of these cells lack the starch and tannins seen with SEM in other neighboring cells; cytoplasmic remnants can be observed with SEM, however (Fig. 4, above).

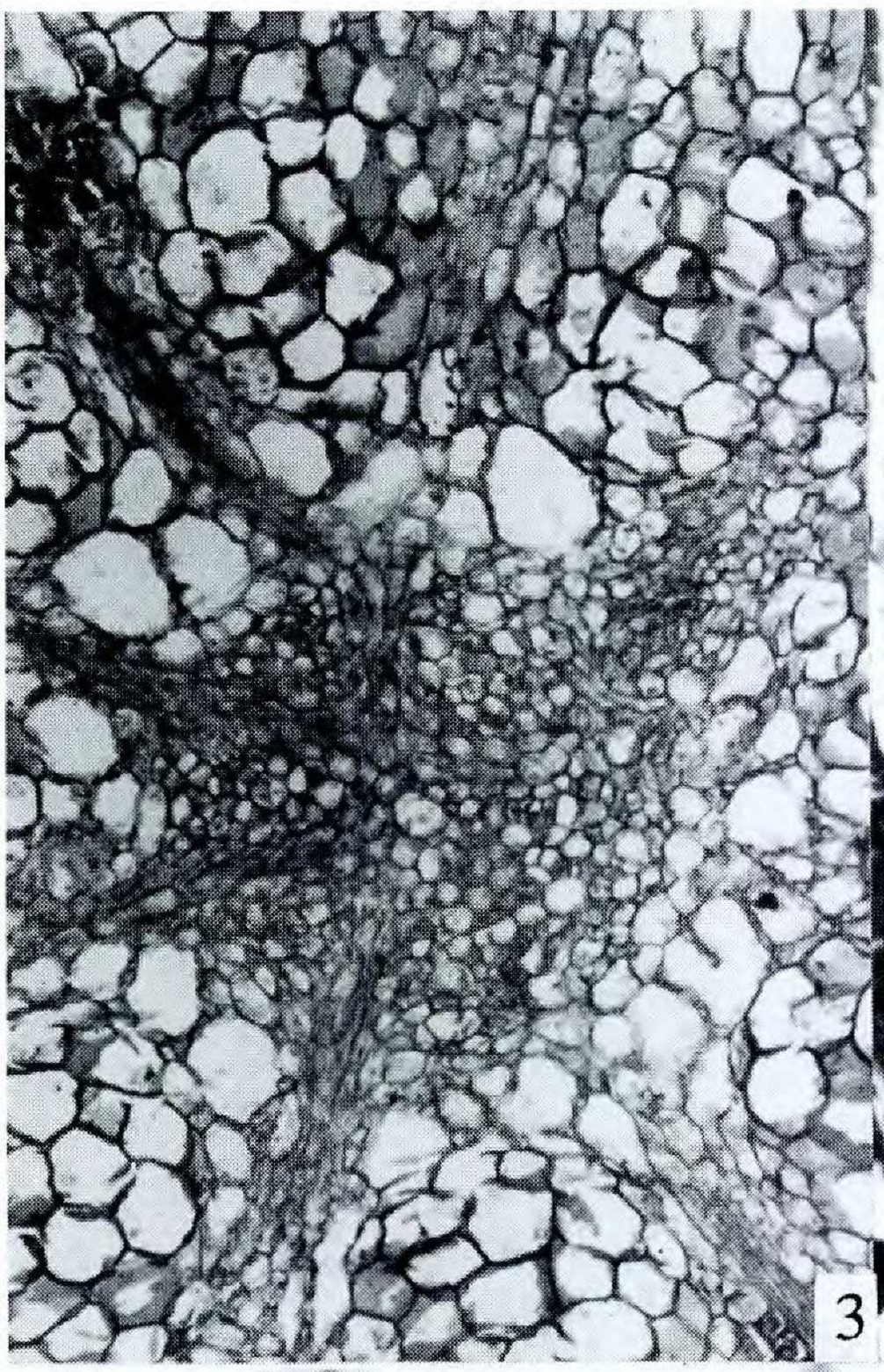
Neither in the main stem nor in the lateral stems did we see the features ordinarily used to identify primary xylem annular, helical, or reticulate bands of secondary wall superimposed on a primary wall. With SEM, one cannot find small pores in primary walls, invisible with light microscopy, that indicate presence of vessels by virtue of the porose walls (e.g., *Barclaya*, Schneider and Carlquist 1995). Xylem can be said to be present only by



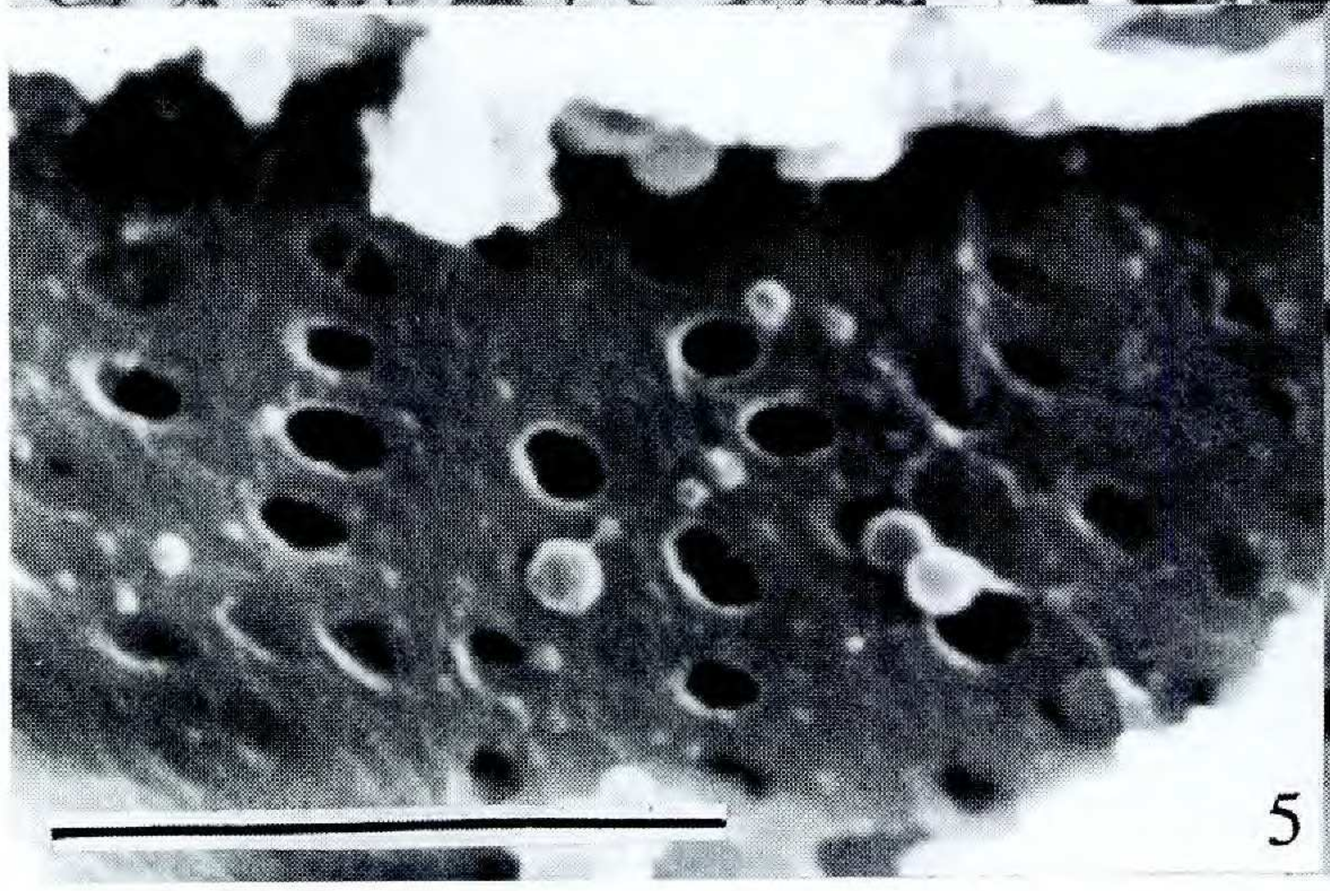

FIGS. 1–2. Sections of mature main stem of *Ceratophyllum demersum*. Fig. 1. Transection of stem, showing cortex delimited from vascular core by a circle of air canals; smaller air canals are evident within the vascular core. Arrows indicate presumptive endodermis between the large air canals. Fig. 2. Median longitudinal section of stem; the main air canals are interrupted at the nodal plate, middle of photograph; smaller air canals adjacent to the nodal plate extend into branches. Figs 1–2, divisions = 10 µm in magnification scales.

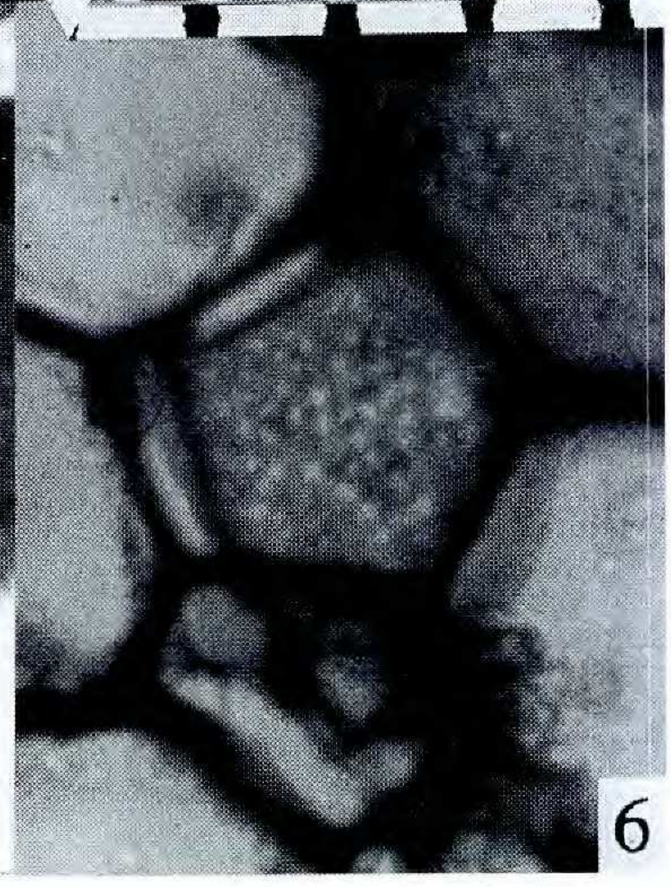
designating the central portion of the core as xylem, as Jones (1931) did, although even he had reservations about doing so. Because neither light microscopy nor SEM reveal any structural characteristics of tracheary elements in this region, we prefer to consider that *Ceratophyllum* lacks xylem.

Sieve-tube elements and companion cells (Figs. 5, 6) are present in the vascular core, usually more commonly around the periphery than in the center. The sieve plates are simple (Figs. 5, 6), with clearly defined pores that are densely placed and more uniform in size than primary pit field pores (Figs. 5, 6). Sieve-tube elments are generally narrower in diameter than the parenchyma cells they accompany, and are not organized into strands or groupings that one expects in phloem strands of stems. For this reason, we do not use the term "phloem parenchyma" as Jones (1931) did. Endress (1994) reports phloem, but no xylem, in floral structures.








FIGS. 3–6. Sections from mature stem of *Ceratophyllum demersum*. Fig. 3. Transection of nodal plate, showing strands of elongate cells extending into bases of lateral branches. Fig. 4. SEM photograph of elongate cells from longisection of lateral branch; remnants of cytoplasm are present on wall surfaces. Fig. 5. SEM photograph of sieve plate of a sieve-tube element from a transection of the main stem. Fig. 6. Sieve tube element (sieve plate in face view) surrounded by parenchyma cells, from transection of vascular core of the main stem. Fig. 4, scale as in Fig. 1; Figs. 4–5, scale bars = 10 μm. Fig. 6, scale divisions = 10 μm.

## DISCUSSION AND CONCLUSIONS

The nature of sieve-tube elements in *Ceratophyllum* is of potential phylogenetic interest, because the sieve-tube elements and their associated companion cells in *Ceratophyllum* correspond to the most specialized phylogenetic condition according to the criteria of Zahur (1959) for woody dicotyledons and Cheadle and Whitford (1941) for monocotyledons. These authors have considered that sieve-tube elements with little differentiation between end wall and lateral wall in morphology of sieve areas, and with no companion cells derived from the same initial as the sieve-tube element, are primitive in dicotyledons and monocotyledons. The genus *Austrobaileya* has been cited as exemplifying this condition (see Metcalfe 1987). However, transverse partitioning in a sieve-tube element may result in end walls bearing simple sieve plates in a dicotyledon with vessels with long scalariform perforation plates in xylem, as in *Cercidiphyllum* (Zahur 1959), and thus sieve-plate morphology may not be a reliable key to degree of phyletic advancement. Also, one can find simple sieve plates with conspicuous pores in peduncles of *Victoria* (Schneider 1976) and other Nymphaeaceae; this may be a byproduct of conductive rates, rather than phyletic status (Carlquist 1975). However, whatever the criteria used, the sieve-tube elements of *Ceratophyllum* do not show a condition that one would call primitive for dicotyledons.

The status of sieve-tube elements is placed first in this discussion because the sieve-tube elements of *Ceratophyllum* do not suggest an incipient stage in the evolution of that cell type. Therefore, one would be tempted to consider the absence of xylem in *Ceratophyllum* to be secondary, a loss or reduction related to adaptation to the submersed habit rather than indicative of a primitive status among angiosperms. If vegetative structures of *Ceratophyllum* are typically submersed, there is little selective value for conduction of water from one part of the plant to another. However, there is, in flowering plants, a selection for phloem because the location of photosynthesizing organs is almost always at a distance from sites for active storage of photosynthates—the fruits (or, in some plants, tubers or other storage organs). Although there is no xylem in the species of *Ceratophyllum*, studied herein there are elongate cells other than sieve-tube elements in the vascular core of stems. Such elongate cells could conceivably serve for moderate degrees of water conduction.

Of prime importance in discussing the probability that xylem was present ancestrally but has been lost is the primary xylem of *Brasenia* (Schneider and Carlquist 1996). In *Brasenia*, annular or helical thickenings of secondary wall material are present at tips of tracheary elements. However, on lateral walls of the tracheary elements of *Brasenia*, these thickenings are reduced to inconspicuous ridges, visible only with SEM, that lack

lignification. *Brasenia* thus offers an example of a stage in reduction of xylary cells with relation to adaptation to the aquatic habitat. Although xylem reduction has been suggested for aquatic plants by a number of authors, likely stages in reduction (other than reduction in quantity of tracheary elements) have not been demonstrated for tracheary tissue of genera other than *Brasenia*.

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